

# **Comparative Study of Lettuce and Radish Grown Under Red and Blue Light-Emitting Diodes (LEDs) and White Fluorescent Lamps**

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## **Abstract**

Growing vegetable crops in space will be an essential part of sustaining astronauts during long-term missions. To drive photosynthesis, red and blue light-emitting diodes (LEDs) have attracted attention because of their efficiency, longevity, small size, and safety. In efforts to optimize crop production, there have also been recent interests in analyzing the subtle effects of green light on plant growth, and to determine if it serves as a source of growth enhancement or suppression. A comparative study was performed on two short cycle crops of lettuce (Outredgeous) and radish (Cherry Bomb) grown under two light treatments. The first treatment being red and blue LEDs, and the second treatment consisting of white fluorescent lamps which contain a portion of green light. In addition to comparing biomass production, physiological characterizations were conducted on how the light treatments influence morphology, water use, chlorophyll content, and the production of ATP within plant tissues.

## 1. Introduction

For the last two decades, NASA's Advanced Life Support project has explored the production of plant crops for space. On long-duration space missions or in extraterrestrial habitations, plants will provide a major source of food, atmospheric regeneration ( $\text{CO}_2 \rightarrow \text{O}_2$ ), water purification, and waste recycling (Yorio *et al.*, 2001). Essentially, the optimization of plant photosynthesis in space relies heavily upon controlling the spectral quality and quantity of light. Previous developments in artificial lighting have led to the steady migration from incandescent sources to fluorescent lamps for improved electrical efficiency. However, if damaged, mercury-containing fluorescent lamps pose significant environmental and health concerns for plants and astronauts (Bourget, 2008).

Recently, more attention has now been given to solid-state lighting with emphasis on light-emitting diodes (LEDs) (Krames *et al.*, 2007). LEDs offer superior efficiency while maintaining miniature sizes, and safer operation without toxic mercury. Furthermore, LEDs have provided several benefits for space applications including low levels of thermal radiation that may heat the plant canopy, no hot electrodes, no high-voltage ballasts, and a very long operating life (~100,000 hours) (Monje *et al.*, 2003; Folta *et al.*, 2005; Massa *et al.*, 2007). In particular, red and blue (RB) LEDs have been useful light sources to drive photosynthesis due to their output near the peak absorption regions of chlorophyll, and the electrical efficiencies are nearly twice that of fluorescent lamps.

Previous studies have shown that various plant species respond differently to certain ratios of light (Goins *et al.*, 1997). In salad crops such as lettuce (*Lactuca sativa*), spinach (*Spinacea oleracea*), and radish (*Raphanus sativas*), Yorio *et al.* (2001) reported that the growth under red LEDs alone was unacceptable. Although improved growth was observed with the addition of 10% blue fluorescent light, their biomass accumulation was optimized under a cool white fluorescent lamp (CWF). This suggests that other wavelengths outside of the red and blue wavebands may be involved in optimizing the growth of salad crops. The supplementation of green light in combination with the RB LEDs has been reported to induce subtle effects on plant growth. The review reported by Folta and Maruhnich (2007) elucidated the fact that plants located in the underbrush of the canopy are significantly limited to the ratios of red and blue light by the overhanging foliage, and since green light is reflected throughout the canopy by plant tissues, it can potentially play a role in photosynthesis under certain conditions (Folta and Maruhnich, 2007). In some instances, green light may function by informing the plant of photosynthetically unfavorable conditions, allowing plants to adjust their compositions and physiology to the available light quality.

Indeed, it has been demonstrated in the literature that plants exhibit different responses when grown under red and blue LEDs alone, when supplemented with green fluorescent lamps, or with CWFs (Kim *et al.*, 2004). However, to the best of our knowledge, there has not been a comprehensive study to compare the response of plants grown under RB LEDs, and plants grown under broad spectrum white fluorescent lamps, which inherently contain a portion of green light. Evaluating the changes in biomass of plants grown under these two treatments may be of great benefit in optimizing crop production for space missions.

### 1.1 Statement of the Problem

To date, the response of various plant crops to spectral quality have been studied by growing them under RB LEDs plus supplemented light from green fluorescent lamps. However, additional studies are required to further evaluate if green light has a direct effect on enhancing



or hindering plant growth. Moreover, due to the low electrical efficiency of green LEDs, the effects of green light were examined herein by growing crops under broad spectrum white fluorescent lamps and compared with identical crops grown in the absence of green light via RB LEDs.

## 2. General Methodology and Procedures

### 2.1. Plant growth chambers and maintenance

The lettuce and radish crops were grown in environmental growth chambers located in the Space Life Sciences Laboratory (SLSL) of NASA Kennedy Space Center. The lettuce and radish seeds were be planted in 10 plastic pots (7 cm tall, 164 mL capacity, four seeds per pot) containing Canadian sphagnum peatmoss. A 1 inch (2.5 cm) layer of sifted arcillite (particle size  $> 2$  mm) was placed at the bottom of each pot to generate a perched water table accessible to plant roots. Within the growth chamber, the pots were arranged inside a  $0.3 \text{ m}^2$  tray under each light treatment. To minimize edge and position effects within the chamber, the pots were rearranged and rotated every other day. The water use was tracked daily by recording water levels in the trays, and recording the amount of water added. The air temperature, relative humidity, and  $\text{CO}_2$  levels were maintained at  $23^\circ \text{C}$ , 70%, and  $1200 \mu\text{mol}\cdot\text{mol}^{-1}$ , respectively.

### 2.2. Light treatments

For the RB treatments, both crops were grown under LED arrays (50-W “UFO” fixtures with 8:1 red:blue ratio) mounted inside the growth chamber. The UFO fixtures are commercially available through AIBC Inc., Ithaca, NY. A photograph of the growth chamber setup is shown in Figure 1 with the RB “UFO” fixtures on the right, and the broad spectrum fluorescent lamp (FL) treatment on the left. The photosynthetically active radiation (PAR) was measured with a Li-Cor quantum meter, and the intensity was maintained at  $\sim 200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  for both crops. The daily photoperiod was arranged to 18 h of active light, and 6 h of no light.



Figure 1. Kennedy Space Center Environmental Growth Chambers with UFO Red and Blue LED treatment (right) and daylight fluorescent tubes treatment (left).

### 2.3. Harvesting and Crop Characterizations

During the crop cycle, each pot was thinned by withdrawing one plant from of each pot on 10, 17, and 25 days after planting. The water use was tracked daily by recording water levels of the holding trays, and recording the amount of water added. During the harvest days, the

morphology of each plant was characterized by measuring shoot length, shoot diameter, fresh weights, and oven-dry weights. The chlorophyll content of the leaves was measured using a SPAD chlorophyll meter, and the total leaf area was measured using a LiCor leaf area meter.

#### 2.4. ATP analysis

When the crops approached maturity at 17 and 25 days after planting, the ATP concentration was analyzed by using leaf tissue extraction procedures. From each plant, 1 gram of leaf tissue from the lettuce and radish, and root tissue from the radish, was measured out and flash frozen in liquid nitrogen. After grinding the frozen tissue, 9 mL of phosphate buffer solution (PBS) was added to make a 1:10 dilution. After stirring and filtering, the supernatant was transferred to micro-channel plate wells where ATP concentration was measured by fluorometric analysis.

### 3. Results and Discussion

The spectral distributions of the two light treatments are illustrated in Figure 2. The red and blue (RB) LEDs exhibit two narrow bands in the blue and red spectral regions with maxima at ~450 and 635 nm, respectively. In contrast, the tri-phosphor fluorescent lamp (FL) treatment consists of multiple sharp bands extending from the UV and through the visible and infrared regions. The presence of green light in the FL treatment is clearly evident by the sharp band occurring at ~546 nm. Likewise, the absence of the 546 nm band in the spectral distribution for the RB LEDs confirms there was no leakage of light from the FL treatment to the side of the growth chamber containing the RB treatment.

Table 1 shows the results of physiological measurements at 25 days after planting (DAP), and are reported as the overall means between cycles 1 and 2. The greatest leaf area was observed for both the lettuce and radish when they were grown under the FL treatment. In like manner, the shoot length and shoot diameters demonstrated a similar trend and were also greater under the FL treatment, potentially indicating an elongation effect on the leaves and stems.

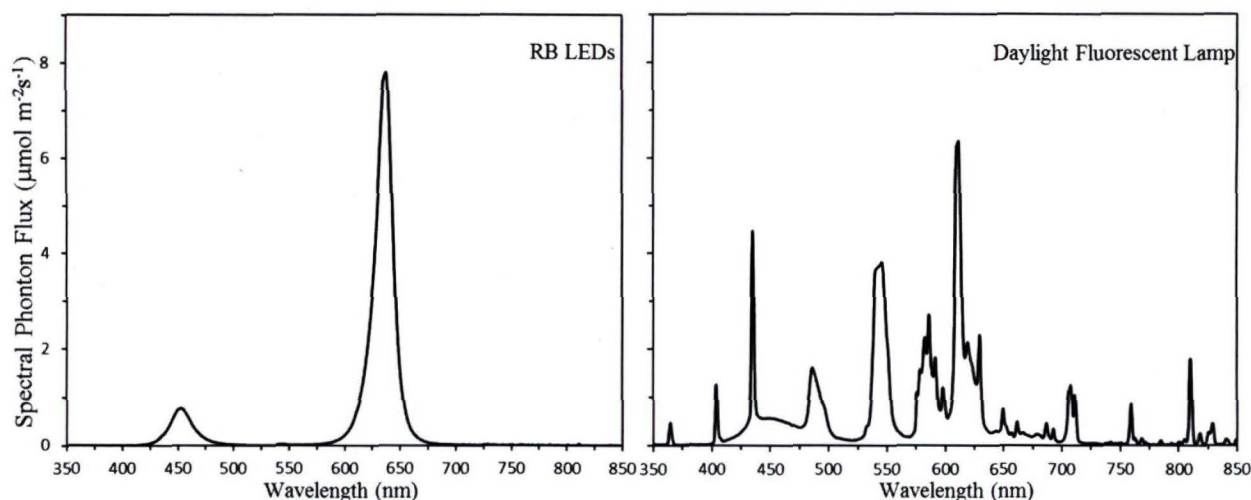


Figure 2. Spectral distributions of light from the red and blue (RB) LEDs treatment (left), and the daylight fluorescent lamp treatment (right). Spectral scans were recorded in the growth chambers with a spectroradiometer.



Table 1. Influence of light quality on leaf area, shoot length, shoot diameter, total fresh weight (FW), total dry weight (DW), and chlorophyll content (Chl) at 25 days after planting (DAP).

Parameter	Lettuce		Radish (leaf)		Radish (root)	
	RB	FL	RB	FL	RB	FL
Leaf area (cm <sup>2</sup> )	330.9	415.5	93.5	135.6	N/A	N/A
Shoot length (mm)	136.5	166.7	115.5	127.1	N/A	N/A
Shoot diameter (mm)	224.9	277.2	213.2	234.1	N/A	N/A
Total FW (g)	15.2	14.4	3.52	4.51	20.1	21.1
Total DW (g)	1.32	0.93	0.53	0.56	1.32	1.23
Chl content (g·m <sup>-2</sup> )	31.3	29.0	49.2	46.6	N/A	N/A

For the lettuce crop, the fresh and dry weight accumulations under the RB treatment were higher. Upon visual inspection, the lettuce grown under the RB light appeared to have thicker leaves and a more waxy-coated texture. Figure 3 portrays the visual comparisons where the dramatic color difference is clearly observed. These significant differences in appearance could suggest an increased production of anthocyanin content within the leaves as a stress response. However, an increase in anthocyanin is a marker for increased flavonoids and anti-oxidants, which could promote a positive consumption benefit for an astronaut crew by counteracting the effects of radiation.

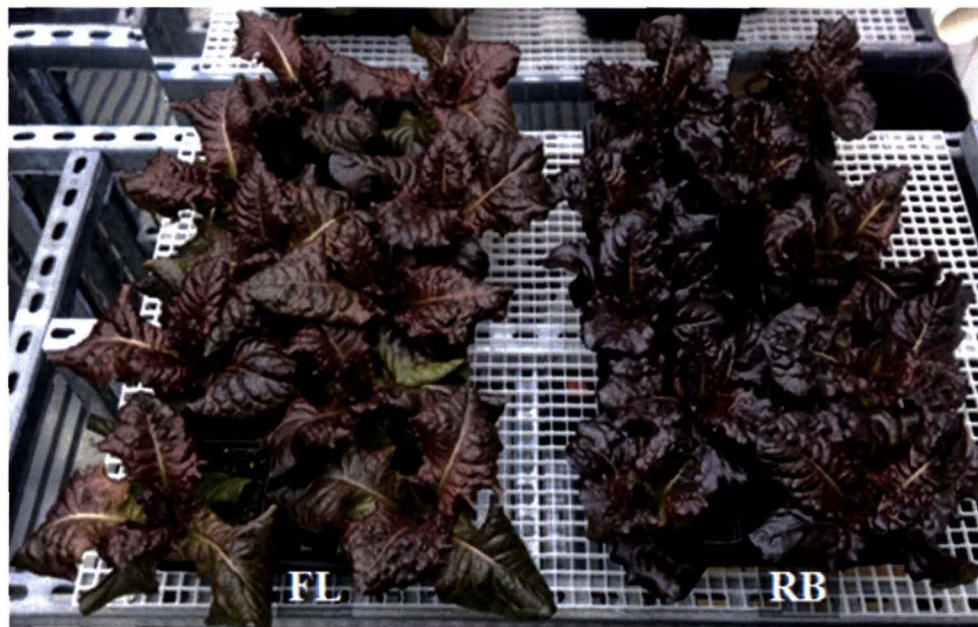


Figure 3. Lettuce crops grown under RB (right) and FL (left) treatments at 25 DAP.

In the case of the radish, the leaf area along with shoot dimensions was also higher under the FL treatment. Unlike the lettuce response, the fresh and dry weight accumulation of the radish leaves were higher under the FL treatment. The fresh and dry weight accumulation of the radish roots appear to be not significantly different. This suggests that the presence or absence



green light on radish root development was not significant, and that mature radish roots could be grown under either RB or FL light treatments. Visually, there was only a minor difference in the radish crops. In Figure 4, the radish exhibit slightly more expanded leaves, which is also supported by the leaf area measurement results shown in Table 1. The SPAD readings indicated that the chlorophyll content of both the lettuce and radish leaves was slightly higher under the RB treatment.

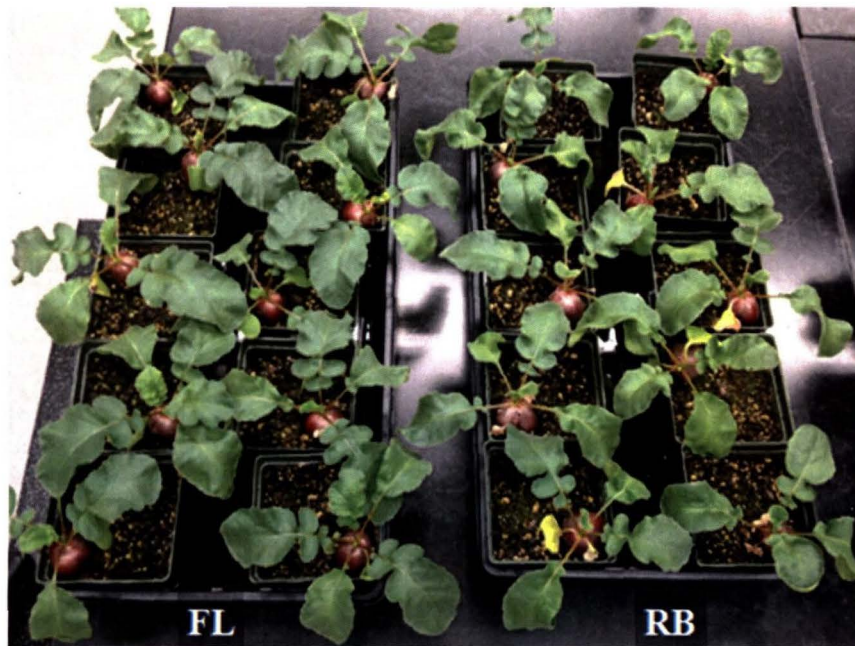


Figure 4. Lettuce crops grown under RB (right) and FL (left) treatments at 25 DAP.

Figure 5 shows the quantities of adenosine triphosphate (ATP) produced by the lettuce leaves at 17 and 25 DAP. The ATP production was analyzed to obtain a clearer understanding of plant metabolism and the management of energy within the cells in response to the RB and FL light treatments. Since ATP is produced as a by-product of glycolysis (breakdown of glucose sugar), knowing its quantity can reveal how much energy is expended for necessary cellular processes such as protein synthesis and tissue growth. In both the RB and FL treatments, the amount of ATP decreases as the lettuce crop reaches maturity, albeit a more dramatic decrease for the FL treatment. Since the morphology of the lettuce grown under the FL treatment revealed larger leaf area and longer shoot dimensions, these results may explain the significantly higher amounts of ATP produced at 17 DAP forecasting the more cellular energy was required to generate elongated tissues.

Figure 6 illustrates the ATP production in the radish leaf and root tissues at 17 and 25 DAP in response to the RB and FL treatments. Unlike the lettuce response, the radish crops exhibited a slight increase in ATP production going from 17 to 25 DAP. In the leaves of the radish, the ATP production was not significantly different between the RB and FL light treatments. However, the ATP accumulation in the radish roots was considerably higher than that of the radish leaves. This observation can be explained by the exponential growth rate and

size of the radish roots near the end of the cycle, while the growth of the leaves changes very little at the end of the crop cycle.

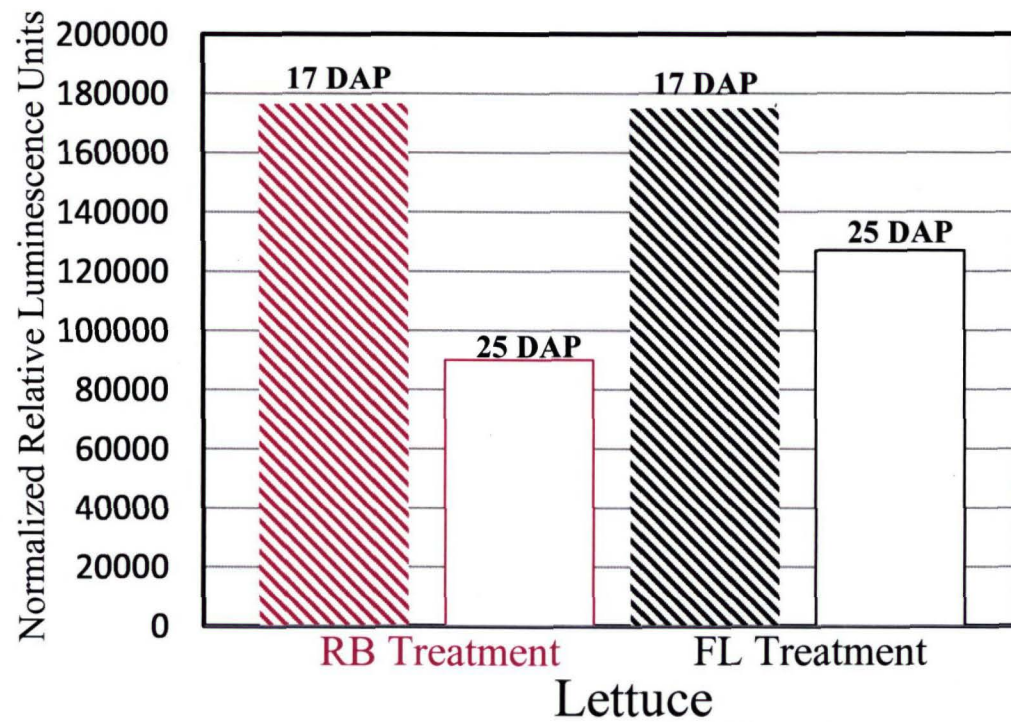


Figure 5. Production of ATP in lettuce leaf tissue in response to RB and FL treatments at 17 and 25 DAP.

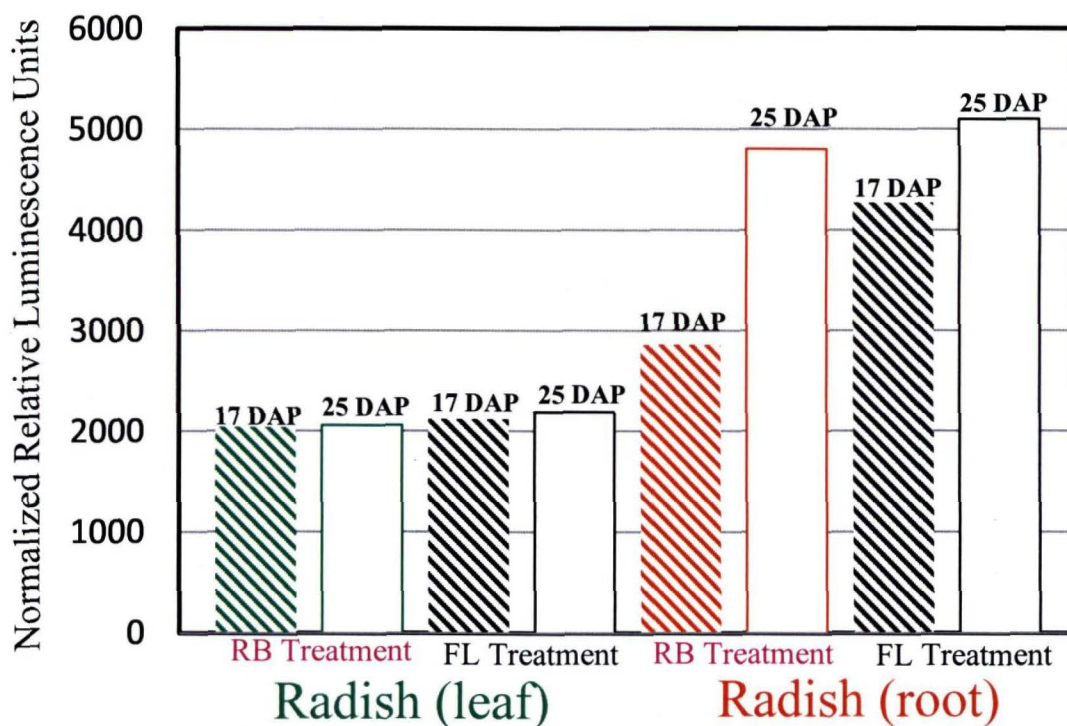


Figure 6. Production of ATP in radish leaf and root tissues in response to RB and FL treatments at 17 and 25 DAP.



#### 4. Conclusion

In summary, lettuce and radish crops were successfully grown under RB and FL light treatments, and their physiological responses recorded and found to exhibit significant differences in some instances. In particular, the plants shoot dimensions and visual appearance. The ATP analysis is a new characterization and will require further study, but has opened new pathways of understanding plant growth. As of now, the project will be replicated for additional cycles in the near future to further confirm the responses that were observed.

#### 5. References

- Bourget, C. M. An introduction to light-emitting diodes. *HortScience*. **2008**, *43*, 1944-1946.
- Folta, K.; Koss, L.; McMorrow, R.; Kim, H.-H.; Kenitz, J. D.; Wheeler, R.; Sager, J. Design and fabrication of adjustable red-green-blue LED light arrays for plant research. *BMC Plant Biol*. **2005**, *5*, 17.
- Folta, K. M.; Maruhnich, S. A. Green light: a signal to slow down or stop. *J. Exp. Bot*. **2007**, *58*, 3099-3111.
- Goins, G. D.; Yorio, N. C.; Sanwo, M. M.; Brown, C. S. Photomorphogenesis, photosynthesis, and seed yield of wheat plants grown under red light-emitting diodes (LEDs) with and without supplemental blue lighting. *J. Exp. Bot*. **1997**, *48*, 1407-1413.
- Kim, H.-H.; Goins, G. D.; Wheeler, R. M.; Sager, J. C. Green-light supplementation for enhanced lettuce growth under red- and blue-light-emitting diodes. *HortScience*. **2004**, *39*, 1617-1622.
- Krames, M. R.; Shchekin, O. B.; Mueller-Mach, R.; Mueller, G. O.; Ling, Z.; Harbers, G.; Craford, M. G., Status and future of high-power light-emitting diodes for solid-state lighting. *J. Display Technol*. **2007**, *3* (2), 160-175.
- Massa, G. D.; Kim, H.-H.; Wheeler, R. M.; Mitchell, C. A. Plant productivity in response to LED lighting. *HortScience*. **2008**, *43*, 1951-1956.
- Monje, O.; Stutte, G. W.; Goins, G. D.; Porterfield, D. M.; Bingham, G. E. Farming in space: Environmental and biophysical concerns. *Adv. Space Res*. **2003**, *31*, 151-167.
- Yorio, N. C.; Goins, G. D.; Kagie, H. R.; Wheeler, R. M.; Sager, J. C. Improving spinach, radish, and lettuce growth under red light-emitting diodes (LEDs) with blue light supplementation. *HortScience*. **2001**, *36*, 380-383.